Biotechnological Interventions for Horticultural Crop Improvement : A Review

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Abstract

The need of fruits and vegetables is growing consistently with the increasing population in the developing countries. How do we keep horticultural production on par with the increasing population? Although conventional plant breeding techniques have made considerable progress in the development of improved varieties, they have not been able to keep pace with the increasing demand for vegetables and fruits in the developing countries. Therefore an instant need is felt to incorporate biotechnology to speed up the crop improvement programmes. Biotechnology has offered wonderful scope and potential to conventional methods of crop improvement, crop protection, crop quality management and other horticultural traits. Biotechnology explores various opportunities in fruit production by providing new genotypes for breeding purpose, supply of healthy and disease free planting material, improvement in fruit quality, enhancing shelf-life, availability of bio- pesticides, bio fertilizers etc. Expression of undesirable genes can be blocked by the application of antisense gene technology and RNAi technology. Eventually Biotechnological interventions that could increase the efficiency of horticultural crop improvement are essential to generate plants with several desirable traits.

Introduction

The requirement of fruits and vegetables is increasing proportionally with the increasing population in the country. How do we keep horticultural production on par with the growing population? Although conventional plant breeding techniques have made considerable progress in the development of improved varieties, they have not been able to keep pace with the increasing demand for vegetables and fruits in the developing countries. Therefore an immediate need is felt to incorporate biotechnology to speed up the crop improvement programmes. Biotechnological tools have revolutionized the entire crop improvement programmes by providing new strains of plants, supply of planting material, more efficient and selective pesticides and improved fertilizers. Many genetically modified fruits and vegetables are already in the market in developed countries. Modern biotechnology encompasses wide areas of biology from utilization of living organisms or substances from those organisms to make or to modify a product, to improve plant or animal or to develop micro-organisms for specific use. It is a new aspect of biological and agricultural science which provides new tools and strategies in the struggle against world's food production problem.

Biotechnology is broadly defined as any technique that uses live organisms viz. bacteria, viruses, fungi, yeast, animal cells, plant cells etc. to make or modify a product, to improve plants or animals or to engineer micro-organisms for specific uses.it encompasses a wide array of techniques through which humans employ biological processes to provide useful products. Biotechnology is essentially and interdisciplinary are consisting of biochemistry, biochemistry, molecular biology,

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microbiology, genetics and immunology etc. Modern biotechnology holds considerable promise to meet challenges in agricultural production. New approaches in biotechnology can develop high yielding and more nutritious crop varieties, improve resistance to disease and also reduce the need for fertilizer and other expensive agricultural chemicals. It could also improve forestry and its products, fiber crops and chemical feed stocks. The major areas of biotechnology which can be adopted for improvement of horticultural crops are:

1. PLANT TISSUE CULTURE : Plant tissue culture can be defined as the culture of all types of plant cells, tissues and organs under aseptic conditions. This definition also extends to the culture of excised embryos and protoplast culture. Plant tissue culture is an integral part of molecular approaches to plant improvement and acts as an intermediary whereby advances made by the molecular biologists in gene isolation and modification are transferred to plant cells. Some of the simpler techniques that are more approachable and have been found to be applied directly in plant propagation and genetic improvement of plants are (i) micropropagation, (ii) meristem culture, (iii) somatic embryogenesis, (iv) somaclonal variation, (v) embryo culture, (vi) *in vitro* selection, (vii) anther culture, and (viii) protoplast culture (**Smith and Drew , 1990).** This technique is economical in time and space affords greater output and provides disease free and elite propagules. It also facilitates safer and quarantined movements of germplasm across nations. When the traditional methods are unable to meet the demand for propagation material this technique can produce millions of uniformly flowering and yielding plants. Micropropagation of almost all the fruit crops and vegetables is possible now. Production of virus free planting material using meristem culture has been made possible in many horticultural crops. Embryo rescue is another area where plant breeders are able to rescue their crosses which would otherwise abort. Culture of excised embryos of suitable stages of development can circumvent problems encountered in post zygotic incompatibility. This technique is highly significant in intractable and long duration horticultural species. In vitro germplasm conservation is of great significance in providing solutions and alternative approaches to overcoming constrains in management of genetic resources. In crops which are propagated vegetatively and which produce recalcitrant seeds and perennial crops which are highly heterozygous seed storage is not suitable. In such crops especially, *in vitro* storage is of great practical importance. These techniques have successfully been demonstrated in a number of horticultural crops and there are now various germplasm collection centers. In vitro germplasm also assures the exchange of pest and disease free material and helps in better quarantine. Micropropagation is the application of tissue culture technique to the propagation of plants starting with very small parts grown aseptically in a test tube or other suitable containers. It is applicable to various crops like ashwagandha **(Bhuria *et al*. 2014)** sugarcane **(Shrivastava *et al*. 2014)** , guava **(Bisen et al, 2014) ,** maize **(Saxena et al.2015).** Deliberate attempts to induce variations in tissue culture have been in progress for the last 60 years and a large number of variants in ornamentals and horticultural crops have been reported **(Rout et al, 2006).** However, there are only a few instances where somaclonal variations have produced agriculturally desirable changes in the progeny. These include sugarcane - increase in cane and sugar yield, and resistance to eye-spot disease **(Larkin and Scowcroft , 1983,)** , potato - improvement of tuber shape, colour and uniformity, and late blight resistance **(Shepherd et al, 1980),** tomato - increased solids, resistance to *Fusarium* race **2 (Evans , 1989)** . There exists a large demand for disease free clones of superior quality plants in ornamental, horticultural, floricultural and agro-forestry sectors. Micropropagation has been carried out in several crop which include, potato, sweet potato, yams, garlic, lime, banana, pineapple and papaya; spices including ginger, small cardamom, turmeric, black pepper and several aromatic and medicinal plants such as sarpgandha and antamul. Elite genotypes of banana, papaya, coconut, small cardamom and oil palm have been multiplied on a commercial scale by private seed companies. Micropropagation of ornamental plants such as gladioli, orchids and bougainvillea which have tremendous export value has been achieved. In the ornamental sector, *Syngonium* provides an excellent example of somaclonal variant where 22 new cultivars, all somaclonal variants, were selected from large populations of tissue-cultured material grown in commercial greenhouses. All 22 cultivars can be traced back to the original 'White Butterfly' clones. Labland Biotech, Mysore has obtained one unique, stable variant of *Spathiphyllum* with golden yellow leaves that has not been found in any Spathiphyllum varieties so far.

Genetic Fidelity : The commercial multiplication of a large number of diverse plants species represents one of the major success stories of utilizing tissue culture technology

profitably. However, a major problem often encountered with the use of tissue culture techniques such as SE is the occurrence of somaclonal variation, which is often heritable as it represents induced genetic changes **(Svabova & Lebeda 2005).** Thus, genetic fidelity testing is an important prerequisite for in vitro regeneration protocols of many crop species, particularly if the resultant plants are to be transplanted to the field**.** Several strategies have been employed to assess the genetic fidelity of regenerated plants, each with their own advantages and disadvantages. Molecular markers facilitate the screening of SE regenerated plants with high precision, and since these markers are unaffected by environmental factors (that can alter phenotypes), they produce reliable and reproducible results. However, for an effective analysis of the genetic stability of in vitro regenerated plantlets, a combination of markers that amplify different regions of the genome should be used **(Alizadeh & Singh 2009; Liu & Yang 2012).**

2. MARKER ASSISTED SELECTION (MAS) : Molecular marker technology is an integral part of Agriculture Biotechnology. PCR based molecular markers are considered as boon for development of agriculture. **(Singh Yogendra, 2010).** Due to ample applications of Agriculture Biotechnology, it is considered as a weapon for scientist to fight hunger, malnutrition and poverty. **(Singh Yogendra, 2009) .**Actually the science of plant genetics traces back to Mendel's classifical studies on garden peas. Since then researchers have been identifying, sorting and mapping single gene markers in many species of higher plants. The markers on first genetic map were phenotypic traits scored by visual observation of morphological characteristics of the flies. Generally a marker must be polymorphic that is it must exist in different forms so that the chromosome carrying the mutant gene can be distinguished from the chromosome with normal gene by the form of marker it also carries. This polymorphism in the marker can be detected at three levels i.e phenotypic level (by Morphological markers), difference in proteins (by Biochemical markers) or difference in the nucleotide sequence of DNA (by Molecular markers).Marker assisted selection or marker aided selection (MAS) is a process whereby a marker (morphological, biochemical or one based on DNA/RNA variation) is used for indirect selection of a genetic determinant or determinants of a trait of interest (e.g. productivity, disease resistance, abiotic stress tolerance, and quality).Considerable developments in biotechnology have led breeders of plants to develop more efficient selection systems to replace traditional phenotype-based selection

Table 1. : Identification of vegetable crop varieties by molecular markers

S. No.	Crop	Technique	Reference
1	Brinjal	RAPD	**Karihaloo et al (1995)**
2	Vegetable Brassica	RAPD	**Cansian and Echeverrigaray (2000)**
3	Tomato	Microsatellites, RAPD, RFLP	**Kaemmer *et al* (1995), Bredemeijer *et al* (1998), Noli *et al* (1999)**
4	Potato	AFLP, microsatellites,ISSR, RAPD	**McGregor *et al* (2000), Ashkenazi *et al* (2001)**
5	Onion, garlic and related species	AFLP, RAPD, SSR, ISSR,	**Arifin *et al* (2000), Fiseher & Bachmann (2000)**
6	Pepper	AFLP, RAPD	**Heras *et al.,* (1996), Paran *et al* (1998)**
7	Beans	RAPD, RFLP	**Stockton and Gepts (1994)**
8	Spinach	Microsatellites	**Groben and Wricke (1998)**
9	Asparagus	RAPD	**Khandka *et al* (1996)**
10	Carrot, Sweet potato	AFLP, RAPD	**Shim and Jorgensen (2000), He *et al* (1995)**
11	Lettuce	AFLP, Microsatellites	**Hill *et al* (1996)**
12	Cucurbits	ISSR, RAPD microsatellites,	**Gwanama *et al* (2000), Danin *et al* (2001)**

systems. While genetically-engineered plants have gained much public attention, another modern breeding technique called MAS (marker-assisted selection) has gone through a silent revolution in recent years. MAS are a technique that does not replace traditional breeding, but can help to make it more efficient. It does not include the transfer of isolated gene sequences such as genetic engineering, but offers tools for targeted selection of the existing plant material for further breeding. The assumption is that the marker used for selection associates at high frequency with the gene or quantitative trait locus (QTL) of interest, due to genetic linkage (close proximity, on the chromosome, of the marker locus and the disease resistance-determining locus)

Salient requirement for MAS : The success of a marker-based breeding depend mainly on three important factors:

- u A genetic map with an adequate number of uniformly spaced polymorphic markers to accurately locate desired quantitative trait loci (QTLs) or major genes.
- u Close linkage between the QTL or a major gene of interest and adjacent markers.
- u Adequate recombination between the markers and the rest of the genome.

A No. of molecular markers involved in horticultural crop improvement are listed in **table 01**

Provitamin A enhanced sweet potato: Provitamin A enhanced sweet potato Sweet potato, a staple food for many people in Africa, has also been suggested as a candidate crop for bio-fortification approaches aimed at alleviating vitamin A deficiency. Traditionally, Africa's predominant sweet potato varieties are white- or yellow-fleshed varieties containing no or only small amounts of provitamin A. However, substantial levels of provitamin A can be found in many orange-fleshed varieties of sweet potato and data show that regular consumption of these varieties does improve vitamin A status (**Tanumihardjo et al. 2008).** In 2001, the International Potato Centre (CIP), together with about 40 other organisations, launched the Vitamin A Partnership for Africa (VITAA) . Since then, the VITAA programme coordinates efforts of local plant breeders to select orange-fleshed varieties with enhanced levels of provitamin A using conventional methods and to promote their increased adoption by farmers in Africa **(Johns & Eyzaguirre 2007).** Today, bio-fortified sweet potato varieties are being disseminated in Africa. The VITAA programme has been most effective in Uganda, Kenya, Mozambique and Tanzania.

MAS have already proven to be a valuable tool for plant breeders. It requires less investment, raises fewer safety concerns, respects species barriers, and is accepted by the public. MAS can be more efficient, effective and reliable than phenotypic selection. Furthermore, MAS can shorten the development time of varieties significantly, so in some cases it will be more cost effective than selection based on phenotypes. MAS also allow the breeding of complex traits not feasible through previous conventional methods. Although certainly not the silver bullet for all problems, MAS is a promising approach to conventional plant breeding.

3. GENETIC ENGINEERING : The transfer of genes from one organism to another is a natural process that creates variation in biological traits. It under lies all attempts to improve agricultural species whether through traditional agricultural breeding or through the techniques of molecular biology. The molecular biological methods of gene transfer alleviate the process to manipulated one gene at a time. They can also control the way in which these genes express themselves in the new variety of plant and animal. This can shorten the time required to develop new varieties and give greater precision. This can also be used to exchange genes. Recombinant DNA manipulation technology is the construction of a stretch of DNA sequence consisting of components derived from different sources. Genetic Engineering involves three major steps :

(i) Identification and isolation of suitable genes for transfer

ii) Delivery system to insert desired gene into recipient cells.

iii) Expression of new genetic information in recipient cells.

Using techniques of genetic engineering many useful genes have been introduced into horticultural plants and many transgenic plants have been developed in which the foreign DNA has been stably integrated and resulted in the synthesis of appropriate gene product.

I. Herbicide tolerance : Transgenic plants are developed that are resistant to herbicides allowing farmers to spray crops so as to kill only weeds but not their crops. Many herbicide tolerant plants have been developed in tomato, tobacco, potato, soybean, cotton, corn oilseed rape, petunia, etc. Glyphosate is one of the most potent broad spectrum environment friendly herbicide known, it is marketed under the trade name Round up. Glyphosate kills plants by blocking the action of an enzyme (5-enolpyruvyl shikimate-3-phosphate synthase)

(EPSPS) an essential enzyme in the biosynthesis of aromatic amino acids, tyrosine, phenylalanine and tryptophan. Amino acids are building blocks of protein. Transgenic plants resistant to Glyphosate have been developed by transferring gene of EPSPS that over produce this enzyme thus inhibiting the effect of Glyphosate. A chimeric EPSP Synthase gene was constructed with the use of the cauliflower mosaic virus 35S promoter to attain high level expression of EPSP Synthase and introduced into petunia cells. Transformed petunia cells as well as regenerated transgenic plants were tolerant to glyphosate **(Shah et al., 1986).** Transgenic pineapple plants transformed with the bar gene for bialaphos resistance were developed **(Sripaoraya et al., 2006)**

II. Engineering pathogen resistance : One of the major constraints limiting the production of fruit crops is diseases caused by several fungi, bacteria and viruses. Viruses are the major pests of crop plants which cause considerable yield losses. Many strategies have been applied to control virus infection using coat protein and satellite RNA. Use of viral coat protein as a transgene for producing virus resistant plants is one of the most spectacular successes achieved in plant biotechnology.Using biotechnological interventions; the coat protein gene of the virus has been transferred to papaya to confer PRSV resistance. Since 1998, GM papayas have been cultivated in Hawaii, USA, which had shown considerable resistance to PRSV. PRSV resistant transgenic papaya varieties 'SunUp' and 'Rainbow' have now occupied >80 % shelf-space in the US market. **Praveen et al. (2010)** developed transgenic plants of tomato with AC4 gene-RNAi construct and the transgenic plants were found to show the suppression of tomato leaf curl virus activity. **Yu et al. (2011)** transformed commercial watermelon cultivars with an untranslatable chimeric construct containing truncated Zucchini Yellow Mosaic Virus coat protein (CP) and Papaya Ring Spot Virus W CP genes. Using a hairpin RNA gene silencing strategy, transgenic poinsettia plants resistant to Poinsettia Mosaic Virus have been developed **(Clarke et al., 2008).**

III. Stress resistance : A number of genes responsible for providing resistance against stresses such as to water stress heat, cold, salt, heavy metals and phytohormones have been identified. Resistance against chilling was introduced into tobacco plants by introducing gene for glycerol-1-phosphate acyl-transferase enzyme from *Arabidopsis.* Many plants respond to drought stress by synthesizing a group of sugar derivatives called polyols (Mannitol, Sorbitol and Sion).Plants that have more polyols are more resistant to stress. Using a bacterial gene capable of synthesizing mannitols it is possible to raise the level of mannitol very high making plants resistant to drought. **Husaini and Abdin (2008)** over-expressed tobacco osmotin gene in strawberry (Fragaria x ananasa Duch.) and found that the transgenic strawberry plants exhibited tolerance to salt stress. **Subramanyam et al. (2011)** expressed tobacco osmotin gene in Capsicum annum and the transgenic chilli plants exhibited improved salt tolerance.

IV. Fruit Quality : Tomatoes which ripen slowly are helpful in transportation process. Transgenic tomato with reduced pectin methyl esterase activity and increased level of soluble solids and higher pH increases processing quality. Tomatoes exhibiting delayed ripening have been produced either by using antisense RNA against enzymes involved in ethylene production (Eg ACC synthase) or by using gene for deaminase which degraded l-aminocyclopropane-l-carboxylic acid (ACC) an immediate precursor of ethylene. This increases the shelf life of tomatoes. Tomatoes with elevated sucrose and reduced starch could also be produced using sucrose phosphate synthase gene. Starch content in potatoes has been increased by 20-40% by using a bacterial ADP glucose pyrophosphorylase gene.

V. Pest resistance : The insecticidal beta endotoxin gene (bt gene) has been isolated from *Bacillus thuringiensis* the commonly occurring soil bacteria and transferred to number of plants like tomato, potato etc. to make them resistant to attack by insects. These genes produce insecticidal crystal proteins which affect a range of lepidopteran, coleopteran , dipteran insects. These crystals upon ingestion by the insect larva are solubilised in the highly alkaline midgut into individual protoxins which vary from 133 to 136 kDa in molecular weight. Insecticidal crystal protein produced during vegetative growth of the cells (VIP)are also found to be highly effective against insect control. Bt resistant plants are already in the market.

Regulations

A number of technical, economic, regulatory and market factors have combined to create hurdles for the utilization of biotechnology in horticultural crops **(Bradford and Alston, 2004).**These includes Species Diversity, Multiple Niche Markets, Small Production/Market Windows per Cultivar, Requirements of Processors, Requirements of Distributors and Retailers, Benefits to Growers, Processors, Distributors and Consumers. Genetically

engineered flowers have been well accepted in the marketplace for over a decade **(Tanaka and Brugliera, 2013)** . A survey of gardeners in Tennessee indicated a majority were likely or very likely to buy genetically engineered ornamentals **(Klingeman and Babbit, 2006).** Given the ability to apply genetic engineering techniques to ornamentals and the positive acceptance in the marketplace, it is perhaps surprising that so few Genetically Enginneered (GE) ornamental products exist. The reason is due to barriers to commercialization that apply to GE varieties, but not to conventionally-bred ornamental varieties **(Sexton and Zilberman , 2011) .** For ornamentals, which have a much smaller market value than food crops and are normally internationally traded, the cost and difficulty of meeting regulatory requirements is the biggest barrier to commercialization. The costs incurred seeking regulatory approval for a transgenic event of a major crop plant are estimated to be millions of dollars **(Bayer et al 2010) .**Though the cost is lower for an ornamental, as no food safety tests are required, there is still a significant cost, potentially amounting to hundreds of thousands of dollars.

Conclusion and Future Perspective

The intellectual application of biotechnology is well-matched with and has much to contribute to agricultural and environmental sustainability while bringing value to producers, distributors and consumers. However, commercialization of such applications has been largely obstructed to date, and additional research in both scientific and policy grounds is needed to expand opportunities for horticultural biotechnology. Even as the adoption of biotech field crops increases every year, biotech horticultural products are struggling to emerge into the marketplace. There is no shortage of targets and applications, particularly with respect to pest management, where biotech crops could dramatically reduce the high rates of pesticide use in horticulture **(Gianessi, 2004**). However, it appears unlikely that additional biotech traits providing primarily grower benefits (so-called input traits) will be marketed in the near future for most horticultural crops (herbicide-tolerant turf grasses may be an exception). Nutritionally improved horticultural products could appeal to consumers and create demand that would lessen distributor risks. However, most targets for nutritional improvement require metabolic engineering of multiple genes, which will need additional research to achieve. Public institutions have traditionally played a major research role in horticultural crops, and this is also true of horticultural biotechnology. How should they respond to the declining private interest in biotechnology research? It may be appropriate to increase research support in cases where there is a convincing public interest, such as the development of nutritionally enhanced food products.

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